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Research Article

Formulation and evaluation of novel nutraceuticals rich in protein, vitamins, minerals, natural flavors, and steviol glycosides for improving quality of life

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Abstract

For a wide range of people, including malnourished adults (12+) with non-communicable diseases (NCDs), micronutrient deficiency, underweight and overweight, pregnant and lactating women, the current study aimed to present novel nutraceuticals structured according to the Standards of Protein-Rich Concentrated Nutrient Supplementary Foods with Daily Value (DV) on the New Nutrition and Supplement Facts Labels of Food and Drug Administration (FDA), for improving quality of life (QoL). Whey protein concentrate, skim milk powder, vitamins (folic acid, B_{12}, C), minerals (iron, zinc), natural flavors (vanilla, cinnamon, coffee, chocolate), and stevia leaves extract were mixed to provide per serving; 10 g protein (20% DV), 25% DV of elemental iron and zinc, vitamin C, folic acid, vitamin B_{12}, with elemental calcium (25% DV) emerged from milk derivatives. The novel nutraceuticals were subjected to the physicochemical, microbiological, and sensory evaluation. Results showed significant differences ($P<0.05$) among formulations with natural flavors added in different amounts. The serving sizes of the four formulations were different according to the difference in the amount of natural flavor while keeping the same amounts of the other ingredients. Following the chocolate-flavored formulation as the first in order of overall acceptability, were coffee, cinnamon, and vanilla-flavored formulation, according to the sensory evaluation.

Keywords

nutraceuticals, quality of life (QoL), whey protein, skim milk, vitamins, minerals, steviol glycosides, natural flavors

Abbreviations

APC – aerobic plate count; BCAAs – branched chain amino acids; DV – daily value; ES – Egyptian Standards; FDA – Food and Drug Administration; FOB – front of the pack; HRQoL – health-related quality of life; HPLC – high-performance liquid chromatography; HSH – high speed homogenizer; IS – Indian Standard; ISO – International Organization of Standardization; LC-MS/MS – liquid chromatography-tandem mass spectrometry; MPP – metallized polypropylene; NCDs – non-communicable diseases; QoL – quality of life; SMP – skim milk powder; SGs – steviol glycosides; TYMC – total combined yeasts and molds count; WPC – whey protein concentrate

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Introduction

A key factor in reducing the severity of infectious diseases is good nutrition. Stronger immune systems, a decreased risk of non-communicable diseases (NCDs), longer life spans, and better nutrition are all associated to enhanced newborn, child, and maternal health. Malnutrition is prevalent and at an elevated risk for occurring in more than 70% of hospitalized patients. Therefore, nutritional therapy specialists who use functional foods to enhance quality of life (QoL) and lessen the severity of diseases like diabetes, obesity, heart disease, arthritis, hepatitis, and depression should regularly include the prevention and treatment of malnutrition by supplementing the patients' diets with healthy nutraceuticals. In order to ensure that therapeutically useful meals with micro- and macronutrients are given in high-quality, safe, nutritionally dense formulae without contaminants, nutritional therapy is also involved in food processing technologies (Almoselhy 2021a).

A food or component of a food that offers medicinal or health advantages, including the prevention or treatment of disease, was the first definition of "nutraceuticals" given by Stephen L. DeFelice in 1989. It is redefined as a food product or secondary metabolite capable of providing health benefits, to treat or prevent diseases in the medical context (Santini and Novellino 2018; Santos et al., 2022).

Modern lifestyle, genetics, and nutritional overload through high-fat diet (HFD) attributed prevalence and diabetes outcomes with various complications primarily due to obesity in which energy-dense diets frequently affect metabolic health. Adaptation of altered diet systems such as the western diet, Mediterranean diet, low glycemic index diet, relatively low carbohydrate consumption, and vegetarian diet trends to optimize calorie requirements for weight reduction and disease management (Prasad et al. 2022). Wellness, quality of life (QoL), and well-being refer to the positive, personal state that is contrary to illness (Meiselman 2016). Health-related quality of life (HRQoL) is an important aspect of physical, social, and psychological well-being. Advanced diet quality was prospectively related with better quality of life and functional capability (Gopinath et al. 2014). By 2050, the proportion of people in Europe who are 65 and older is predicted to reach an all-time high and make up more than 30% of the population. The quality of life across the lifetime was the focus of the 6th European Conference on Sensory and Consumer Research, where a key issue was the function of food in healthy ageing (Giacalone et al. 2016). Understanding consumer food choices and creating ways to encourage healthy eating habits depend on seeming well-being (Jaeger et al. 2022). Maintaining a healthy diet during pregnancy may be crucial in creating the groundwork for how children will react to food consumption in their early years (Delahunt et al. 2022).

The current study aimed to design and evaluate four nutraceutical formulations in order to establish their validity with the most desirable attributes and consumer acceptance in light of the growing need for high-quality and secure nutraceutical formulations. Physicochemical, microbiological, sensory, and nutrition facts were determined.

Materials and Methods

Materials. All ingredients: whey protein concentrate (WPC 70% protein), skim milk powder (SMP 34% protein), natural flavors of vanilla, cinnamon, instant coffee, and raw dark cocoa, zero calorie sweetener of stevia leaves extract (steviol glycosides), anti-caking agent as silicon dioxide (silica - E551), ferrous fumarate (33% elemental iron), zinc gluconate (13% elemental zinc), ascorbic acid (vitamin C), sodium folate (98% folic acid - vitamin B_9), and cyanocobalamin (10% vitamin B_{12}) were imported from European Union companies.

Chemicals and reagents. All solvents and chemicals used in the study were of analytical and HPLC grade obtained from Sigma-Aldrich, USA.

Formulation of the novel nutraceuticals. The novel nutraceuticals were designed according to the Egyptian Standards (ES:2730/2007) and the Indian Standard (IS: 8220/1976). Experimental design as illustrated in Figure 1. All ingredients as shown in Table 1, were mixed and homogenized using a mechanical high-speed homogenizer (HSH) at 1000 rpm for 2 min, then packaged in sealed metallized polypropylene (MPP) sachets and kept in a cool dry place away from direct sunlight till analysis and sensory evaluation.

Physical characteristics and proximate composition. Appearance, texture, and flavor were determined along with moisture, ash, protein, fat, fiber, and carbohydrate content (by difference)

Figure 1. Experimental design

Table 1. Nutritional composition of the novel nutraceutical formulations per 100 g

	Formula			
Ingredients, %	**Vanilla**	**Cinnamon**	**Coffee**	**Chocolate**
Whey protein concentrate (WPC 70%)	38.3823545	35.0451495	35.0451495	32.242137
Skim milk powder (SMP 34%)	61.094	55.769	55.769	51.288
Natural flavor	0.01	8.696	8.696	16
Steviol glycosides (SGs)	0.2	0.2	0.2	0.2
Silica (E551)	0.04	0.04	0.04	0.04
Sodium folate (98% Folic acid - B_9)	0.000490	0.000448	0.000448	0.000412
Cyanocobalamin (10% Vitamin B_{12})	0.000028	0.000026	0.000026	0.000024
Ascorbic acid (Vitamin C)	0.1071435	0.0978255	0.0978255	0.09
Ferrous fumarate (33% Fe)	0.065238	0.059565	0.059565	0.0548
Zinc gluconate (13% Zn)	0.100746	0.091986	0.091986	0.084627
Total weight of all ingredients (g)	100.00	100.00	100.00	100.00

according to the AOAC Official Method 965.33 (2016) and the total energy (Calories) per 100 g was calculated (Abd El-Baset and Almoselhy 2023a).

Determination of steviol glycosides. Steviol glycosides content was determined according to Vaněk et al. (2001) using HPLC.

Determination of vitamins. Folic acid (vitamin B_9) and vitamin B_{12} were determined according to Salvati et al. (2016) using LC-MS/MS. Whereas, vitamin C was determined according to the AOAC Official Method 967.21 (2012) using HPLC.

Determination of minerals and heavy metals. Minerals (sodium, calcium, iron, and zinc) and heavy metals (lead, cadmium, and arsenic) were determined according to the method described by Sepe et al. (2003). Sodium and calcium were determined using inductively coupled plasma optical emission spectrometry (ICP-OES). Whereas, iron, zinc, lead, cadmium, and arsenic were determined using inductively coupled plasma mass spectrometry (ICP-MS).

Determination of preservatives. Preservatives (benzoates and sorbates) were determined according to Saad et al. (2005) using HPLC.

Chemical migration testing for the packing material MPP. Chemical migration testing was performed on the packing material made from MPP according to Zhang et al. (2022).

Microbiological analysis of the novel nutraceuticals. According to the procedure outlined in ISO 4833-1:2013 (2013), the total combined yeasts and molds count (TYMC) and the aerobic plate count (APC) were performed. Whereas, the detection of *Salmonella* was carried out in accordance with ISO 6579-1:2017 (2017a), the detection of *Bacillus cereus* was carried out in accordance with ISO 7932: 2004 (2004), the detection of *Escherichia coli* was carried out in accordance with ISO 16649-2: 2001 (2001), and the detection of *Listeria species* was carried out in accordance with ISO 11290-1:2017 (2017b).

Determination of aflatoxin and deoxynivalenol in the novel nutraceuticals. Analysis of aflatoxin (B1, B2, G1, G2) and deoxynivalenol was carried out according to Sulyok et al. (2020) using LC-MS/MS.

Sensory evaluation of the novel nutraceuticals. Sensory evaluation performed in this study was carried out with ethical approval and consent to participate in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Thirty trained sensory panelists in the field of food science (15 women and 15 men, ages 25 to 70) were chosen on the basis of voluntariness with the understanding that the samples under evaluation were safe. The only participants in this research had to be regular users of this kind of products. Participants who had a milk, flavor, or sweetener allergy were not permitted to join. A nine-point Hedonic scale, which indicates the degree of participants' general liking or disliking for quality characteristics such as appearance, aroma, taste, texture, and acceptability, was used to ask participants to rate the products. According to the evaluator, each attribute's score ranged from 1 to 9 (1: extremely dislike; 9: extremely like). The average intensity score for each attribute was determined and tallied when the evaluation process was finished. The samples were given to the panelists in the form of powdered nutraceutical formulations in sealed sachets labeled with the four flavors (vanilla, cinnamon, coffee, and chocolate), to be prepared with cold drinking water in disposable plastic cups with spoons. To ensure that the items given to the panelists were of the greatest acceptable quality and safety, the samples were kept for 6 months (the period of chemical and microbiological examination) before the sensory evaluation was conducted.

Nutrition facts of the novel nutraceuticals. Nutrition facts of the nutraceutical formulations were determined from the analytical methods such as proximate composition, minerals, and vitamins compliant with the recommendations of the Food and Drug Administration (FDA 2022).

Statistical analysis. All measurements were performed in three replicates, whereas the sensory evaluation was carried out in 30 replicates. Data were shown as mean ± standard deviation (SD). Analysis of variance (ANOVA) was conducted with the SPSS software at $P<0.05$.

Results and Discussion

Proximate composition and physical characteristics of the novel nutraceuticals. Appearance, texture, and flavor were determined along with moisture, ash, protein, fat, fiber, carbohydrate, and energy per 100 g of the novel nutraceuticals are shown in Table 2. It is noticed the significant differences ($P<0.05$) among different formulations due to the differences in composition because of the different amounts of the natural flavors added, as the nutraceutical formulations were designed according to the specific regulations which recommend labeling the serving unit which differs among the formulations as follows; Vanilla-flavored formulation (21 g); Cinnamon-flavored formulation (23 g); Coffee-flavored formulation (23 g); Chocolate-flavored formulation (25 g), and the proximate composition was evaluated on a 100 g basis of each formulation. Also, the added natural flavors resulted in differences in proximate compositions as some of them have excess plant protein content as in the chocolate-flavored formulation which contains raw dark cocoa powder with plant protein content, fat, carbohydrate, fiber, minerals, and vitamins. Also, the cinnamon-flavored formulation contains carbohydrates and fiber, whereas, the coffee-flavored formulation powder contains carbohydrates exceeding that of the vanilla-flavored formulation.

Steviol glycosides, vitamins, minerals, heavy metals, and preservatives in the novel nutraceuticals. Steviol glycosides, vitamins, minerals, heavy metals, and preservatives in the

novel nutraceuticals were determined and tabulated in Table 3. No significant difference was detected among all samples considering the content of steviol glycosides which was the same in all samples coinciding with the preparation content added in the same amounts per 100 g of formulation. Significance differences ($P<0.05$) among formulations were observed in vitamins and minerals contents due to the difference in composition as the additives were added in different ratios according to the serving units which were 21, 23, 23, 25 g for the vanilla, cinnamon, coffee, and chocolate-flavored formulations, respectively (not per 100 g as in steviol glycosides). Cinnamon-flavored and chocolate-flavored formulations had more contents of iron and zinc due to the richness of cinnamon and cocoa powders in the 2 elements. No heavy metals (lead, cadmium, and arsenic) were detected in any of the samples. Also, no preservatives (benzoates and sorbates) were detected in any of the samples. The results indicate the safety of products for human consumption.

Table 2. Physical characteristics and proximate composition of the novel nutraceutical formulations

	Formula			
Parameter	**Vanilla**	**Cinnamon**	**Coffee**	**Chocolate**
Appearance	Creamy white	Brownish	Brownish	Brownish
Texture	Fine powder	Fine powder	Fine powder	Fine powder
Flavor	Vanilla	Cinnamon	Coffee	Chocolate
Moisture %	4.28 ± 0.20^{d}	4.50 ± 0.12^{b}	4.43 ± 0.09^{c}	4.66 ± 0.12^{a}
Ash %	4.45 ± 0.03^{d}	4.87 ± 0.00^{b}	4.54 ± 0.06^{c}	4.97 ± 0.03^{a}
Protein %	48.05 ± 0.02^{a}	44.17 ± 0.03^{b}	44.04 ± 0.03^{c}	42.64 ± 0.08^{d}
Fat %	2.85 ± 0.01^{b}	2.61 ± 0.03^{c}	2.61 ± 0.02^{c}	4.00 ± 0.06^{a}
Fiber %	0.00 ± 0.00^{c}	4.60 ± 0.04^{b}	0.00 ± 0.00^{c}	5.28 ± 0.06^{a}
Carbohydrate %	40.37 ± 0.13^{b}	39.25 ± 0.27^{c}	44.38 ± 0.17^{a}	38.45 ± 0.43^{d}
Energy (calories)	379.33 ± 0.38^{a}	366.37 ± 0.63^{d}	377.17 ± 0.44^{b}	370.92 ± 0.85^{c}

Values are mean ± standard deviation (n=3). $^{a-d}$ Means in the same row with different superscripts are significantly different (P<0.05).

Chemical migration testing for the packing material MPP. The concentrations of the measured chemical elements were compared with their migration limits in accordance with the Commission Regulation (EU) 10/2011 on plastic materials and articles intended to come into contact with food, in order to quantify the chemical migration and risk of packing materials. Table 4 displays the results of the chemical migration testing, all of which fell below the limit of detection (LOD), which is set at 0.01 ppm for all elements based on the instruments used. This proves the safety of the packing material made from metallized polypropylene (MPP), which is used in products packaging (Abd El-Baset and Almoselhy 2023b). MPP is a superior food packaging material that protects the superior quality and safety of food products because the aluminum layer used in metallized packing is directly applied onto the polymer surface of the packing material through the vacuum deposition technique without the use of adhesives, which could potentially cause toxic chemicals from the adhesive layer to migrate into the food matrix in laminated multilayer films of packing materials (Zhang et al. 2022).

Microbiological analysis. From Table 5, microbial contamination and the presence of spores were not detected. The maximum values observed for the aerobic plate count (APC) and total combined yeasts and molds count (TYMC) were 0.63 and 0.22 CFU.g^{-1}, respectively. No *Bacillus cereus* and *E. coli* were detected in any sample. *Salmonella spp.* and *Listeria spp.* were absent in all samples. Also, there were significant differences ($P<0.05$) in APC and TYMC between different samples under different types of natural flavors which reflect the effect of natural flavor on the microbiological activity of the product, where the APC values were 0.30, 0.40, 0.53, and 0.63 CFU.g^{-1} in cinnamon, coffee, vanilla, and chocolate-flavored formulations, respectively. The TYMC values were 0.13, 0.15, 0.17, and 0.22 CFU.g^{-1} in cinnamon, coffee, vanilla, and chocolate-flavored formulations, respectively, demonstrating apparent

Table 3. Determination of steviol glycosides, vitamins, minerals, heavy metals, and preservatives in the novel nutraceutical formulations

	Formula			
Parameter	**Vanilla**	**Cinnamon**	**Coffee**	**Chocolate**
Sweetener				
Steviol glycosides mg.kg^{-1}	2004±2^{a}	2000±3^{a}	2001±1^{a}	2003±1^{a}
Vitamins				
Folic acid (Vitamin B_9) µg.kg^{-1}	4823±13^{a}	4400±24^{c}	4402±10^{b}	4084±4^{d}
Cyanocobalamin (Vitamin B_{12}) µg.kg^{-1}	29±5^{a}	27±6^{b}	27±4^{b}	26±2^{c}
Ascorbic acid (Vitamin C) mg.kg^{-1}	1072±5^{a}	984±7^{b}	980±2^{c}	913±1^{d}
Minerals				
Sodium mg.kg^{-1}	1192±5^{a}	1088±9^{b}	1087±8^{b}	1002±12^{c}
Calcium mg.kg^{-1}	15483±17^{a}	14133±13^{b}	14135±11^{b}	13027±16^{c}
Iron mg.kg^{-1}	216±5^{c}	280±4^{a}	196±4^{d}	256±3^{b}
Zinc mg.kg^{-1}	131±8^{c}	186±6^{a}	124±6^{d}	175±7^{b}
Heavy metals				
Lead mg.kg^{-1}	ND	ND	ND	ND
Cadmium mg.kg^{-1}	ND	ND	ND	ND
Arsenic mg.kg^{-1}	ND	ND	ND	ND
Preservatives				
Benzoates (as Benzoic acid) mg.kg^{-1}	ND	ND	ND	ND
Sorbates (as Sorbic acid) mg.kg^{-1}	ND	ND	ND	ND

ND: Not detected. Values are mean ± standard deviation (n=3). $^{a-d}$ *Means in the same row with different superscripts are significantly different (P<0.05)*

superiority of cinnamon as an antimicrobial followed by coffee, vanilla, and chocolate flavor. The detected values of the microbiological activity in all samples were lower than the permitted range stipulated by the Commission Regulation (EC) 2073/2005.

Aflatoxin and deoxynivalenol analyses in the novel nutraceuticals. Determination of Aflatoxin and deoxynivalenol is considered a routine analysis in food samples with the emergence of mycotoxins in dairy products as a result of the contamination of the milk by the contaminated feeding stuff consumed by the cows or by the direct fungal contamination of the dairy products which may result in the formation of mycotoxins (Becker-Algeri et al. 2016; Flores-Flores and González-Peñas 2018; Van Egmond 1983). From the results shown in Table 6, for aflatoxin (B1, B2, G1, G2) and deoxynivalenol, the obtained values did not reach the limit of quantitation (LOQ) by the analytical instrument which has been assigned as 0.2 µg.kg^{-1} for aflatoxins and 50 µg.kg^{-1} for deoxynivalenol. These values (LOQ) were considerably less than the ranges permitted by the official regulations. Thereby, all the tested samples of the nutraceutical formulations with different types of natural flavors were safe for consumption as the aflatoxins and deoxynivalenol values did not reach the benchmark values assigned by Commission Regulation (EU) 165/2010 for aflatoxins and Commission Recommendation (EC) 2006/576 for deoxynivalenol. Also, there were no significant differences between different samples under different types of flavors, and hence, there was no effect of flavor on the contents of aflatoxins and deoxynivalenol which indicated the absence of any contamination during the processing of products.

Table 4. Chemical migration testing for the packing material (metallized polypropylene - MPP)

Chemical elementes, ppm	Packing material (metallized polypropylene - MPP)	
	After soaking in 10% ethyl alcohol for 20 d at room temperature	After soaking in 3% acetic acid for 20 d at room temperature
Copper (Cu)	<LOD	<LOD
Zinc (Zn)	<LOD	<LOD
Manganese (Mn)	<LOD	<LOD
Iron (Fe)	<LOD	<LOD
Cobalt (Co)	<LOD	<LOD
Barium (Ba)	<LOD	<LOD
Lithium (Li)	<LOD	<LOD

LOD – Limit of detection (0.01 ppm)

Table 5. Microbiological analysis of the novel nutraceutical formulations

Microbe	Formula			
	Vanilla	Cinnamon	Coffee	Chocolate
Aerobic plate count APC, CFU.g^{-1}	0.53±0.03^{b}	0.30±0.01^{d}	0.40±0.02^{c}	0.63±0.04^{a}
Yeasts and molds count CFU.g^{-1}	0.17±0.02^{b}	0.13±0.01^{d}	0.15±0.01^{c}	0.22±0.02^{a}
Bacillus cereus CFU.g^{-1}	Absent	Absent	Absent	Absent
E. coli (CFU.g^{-1}	Absent	Absent	Absent	Absent
Salmonella spp., presence per 25 g	Absent	Absent	Absent	Absent
Listeria spp., presence per 25 g	Absent	Absent	Absent	Absent

Values are mean ± standard deviation (n=3). $^{a-d}$ Means in the same row with different superscripts are significantly different (P<0.05).

Table 6. Determination of aflatoxin and deoxynivalenol in the novel nutraceutical formulations

Compound	Formula			
	Vanilla	Cinnamon	Coffee	Chocolate
Aflatoxin B1	<LOQ	<LOQ	<LOQ	<LOQ
Aflatoxin B2	<LOQ	<LOQ	<LOQ	<LOQ
Aflatoxin G1	<LOQ	<LOQ	<LOQ	<LOQ
Aflatoxin G2	<LOQ	<LOQ	<LOQ	<LOQ
Deoxynivalenol	<LOQ	<LOQ	<LOQ	<LOQ

LOQ – Limit of quantitation (0.2 µg/kg for Aflatoxin B1, B2, G1, G2 and 50 µg/kg for Deoxynivalenol)

Sensory evaluation. Sensory evaluation of the nutraceutical formulations is an important step to knowing the consumer's perception of the final product. The sensory attributes of appearance, aroma, taste, texture, and overall acceptability were evaluated ($P<0.05$) as being in the range of 1 to 9. As shown in Table 7, there were significant differences ($P<0.05$) in all sensory attributes. In terms of appearance, taste, and texture, the chocolate-flavored formulation was the best followed by coffee, cinnamon, and vanilla with the last position despite the vanilla-flavored formulation being the best in the aroma followed by a cinnamon, coffee, and chocolate-flavored formulation which reflects the apparent effect of changing natural flavors. The chocolate-flavored formula was the best acceptable according to the sensory evaluation scores, followed by coffee, cinnamon, and vanilla-flavored formulations.

Table 7. Sensory evaluation of the novel nutraceutical formulations

Sensory attribute	Formula			
	Vanilla	**Cinnamon**	**Coffee**	**Chocolate**
Appearance	8.23 ± 0.21^{d}	8.73 ± 0.38^{c}	8.82 ± 0.03^{b}	8.92 ± 0.07^{a}
Aroma	8.72 ± 0.03^{a}	8.43 ± 0.05^{b}	8.20 ± 0.05^{c}	8.06 ± 0.04^{d}
Taste	8.25 ± 0.05^{d}	8.28 ± 0.08^{c}	8.42 ± 0.06^{b}	8.76 ± 0.13^{a}
Texture	8.24 ± 0.06^{d}	8.33 ± 0.06^{c}	8.54 ± 0.10^{b}	8.90 ± 0.10^{a}
Overall acceptability	8.36 ± 0.09^{d}	8.44 ± 0.14^{c}	8.50 ± 0.06^{b}	8.66 ± 0.09^{a}

Values are mean ± standard deviation (n=30). $^{a\text{-}d}$ *Means in the same row with different superscripts are significantly different (P<0.05).*

Nutrition facts of the novel nutraceuticals. Nutrition facts (Figure 2) are considered a mandatory act for labeling dietary supplements with essential nutrients including fat, carbohydrate, dietary fiber, protein, minerals, vitamins, and calories per serving for health purposes. 2,000 calories a day is used for general nutrition advice (FDA 2022). Considering the nutrition facts information provided on the outer package, the labeled food product is better than the unlabeled. The labeling of food products as containing "natural" ingredients creates a potent marketing tool, as consumers pay more for natural products that express naturalness and being a better and more safe product (Hartmann et al. 2018). To endorse healthier eating behaviors, regulatory authorities around the world encourage food labeling with mandatory basic nutrition information on the front of the pack (FOP) with the calorie and nutrition information already provided. The most effective nutrition label increases promotion, marketing, and sales of foods (Dubois et al. 2021). The Five-Color Nutrition Label based was effective in promoting healthier food products (Ducrot et al. 2016).

Figure 2 shows significant differences among all products considering the energy per serving. The chocolate-flavored formulation had the highest energy as 90 calories followed by coffee (86 calories), cinnamon (84 calories), and vanilla with the least energy (79 calories) due to different compositions of the added flavors, as the raw dark cocoa contains excess dietary fiber, carbohydrate, fat, and plant protein with increased values. Cinnamon had dietary fiber and carbohydrates, and instant coffee had carbohydrates. All products showed good stablility with recommended shelf life of 18 months from production date.

All formulations were low-calorie without any vegetable fat added from refined edible oils (as many products have a considerable vegetable fat content as a source of energy) to avoid the processing contaminants emerging in the refined edible oils (Almoselhy 2021b; Almoselhy et al. 2021). All products were free from added sugars or

Vanilla-Flavored Formulation

Nutrition Facts

10 Servings Per Container
Serving Size 1 Sachet [21 gm]

Amount Per Serving Calories 79

		% Daily Values *
Total Fat 0.6 g		**< 1%**
Saturated Fat 0.25 g		1.3%
Trans Fat 0 g		0%
Cholesterol 0 mg		**0%**
Sodium 25 mg		**1.1%**
Total Carbohydrate 8.5 g		**3.1%**
Dietary Fiber 0 g		0%
Total Sugars 8.5 g		
Includes 0 g Added Sugars		0%
Protein 10 g		**20%**
Folic acid	100 mcg	25%
Vitamin B_{12}	0.6 mcg	25%
Vitamin C	22.5 mg	25%
Calcium	325 mg	25%
Iron	4.5 mg	25%
Zinc	2.75 mg	25%

* % Daily Values based on a 2000 calories diet.

Cinnamon-Flavored Formulation

Nutrition Facts

10 Servings Per Container
Serving Size 1 Sachet [23 gm]

Amount Per Serving Calories 84

		% Daily Values *
Total Fat 0.6 g		**< 1%**
Saturated Fat 0.25 g		1.3%
Trans Fat 0 g		0%
Cholesterol 0 mg		**0%**
Sodium 25 mg		**1.1%**
Total Carbohydrate 10.1 g		**3.7%**
Dietary Fiber 1 g		3.6%
Total Sugars 9.1 g		
Includes 0 g Added Sugars		0%
Protein 10 g		**20%**
Folic acid	100 mcg	25%
Vitamin B_{12}	0.6 mcg	25%
Vitamin C	22.5 mg	25%
Calcium	325 mg	25%
Iron	4.5 mg	25%
Zinc	2.75 mg	25%

* % Daily Values based on a 2000 calories diet.

Coffee-Flavored Formulation

Nutrition Facts

10 Servings Per Container
Serving Size 1 Sachet [23 gm]

Amount Per Serving Calories 86

		% Daily Values *
Total Fat 0.6 g		**< 1%**
Saturated Fat 0.25 g		1.3%
Trans Fat 0 g		0%
Cholesterol 0 mg		**0%**
Sodium 25 mg		**1.1%**
Total Carbohydrate 10.2 g		**3.7%**
Dietary Fiber 0 g		0%
Total Sugars 10.2 g		
Includes 0 g Added Sugars		0%
Protein 10 g		**20%**
Folic acid	100 mcg	25%
Vitamin B_{12}	0.6 mcg	25%
Vitamin C	22.5 mg	25%
Calcium	325 mg	25%
Iron	4.5 mg	25%
Zinc	2.75 mg	25%

* % Daily Values based on a 2000 calories diet.

Chocolate-Flavored Formulation

Nutrition Facts

10 Servings Per Container
Serving Size 1 Sachet [25 gm]

Amount Per Serving Calories 90

		% Daily Values *
Total Fat 1 g		**1.3%**
Saturated Fat 0.4 g		2%
Trans Fat 0 g		0%
Cholesterol 0 mg		**0%**
Sodium 25 mg		**1.1%**
Total Carbohydrate 10.9 g		**4%**
Dietary Fiber 1.3 g		5%
Total Sugars 9.6 g		
Includes 0 g Added Sugars		0%
Protein 10 g		**20%**
Folic acid	100 mcg	25%
Vitamin B_{12}	0.6 mcg	25%
Vitamin C	22.5 mg	25%
Calcium	325 mg	25%
Iron	4.5 mg	25%
Zinc	2.75 mg	25%

* % Daily Values based on a 2000 calories diet.

Figure 2. Nutrition facts of vanilla, cinnamon, coffee, and chocolate flavored formulations

animal fats. The total sugars contents appearing in Nutrition Facts were calculated from the milk sugar (lactose) which is present in skim milk powder (SMP) and whey protein concentrate (WPC).

Novelty impact statement and health benefits of the novel nutraceuticals. Novelty of the study can be summed up as a new concept for formulation of nutraceuticals on a scientific basis in accordance with the mandatory registration regulations, with updated DV according to FDA (FDA 2022).

The formulations were free from artificial colors, flavors, fillers, and preservatives to avoid any potential negative effects including allergic reactions, weight gain, gas, flatulence, and bloating. To adhere to the assertions that added sugars should be reduced in a healthy diet, no added sugar was present. More benefits achieved from the improved high-speed homogenization technique (Almoselhy 2022; Azab et al. 2022) and appropriate ingredient choice, starting with the high-quality protein source of WPC 70% and SMP 34% apart from the cheap plant protein such as soy protein, especially with the raised concerns in many studies that the phytoestrogens (isoflavones) in soy may feminize men by lowering testosterone levels and raising estrogen levels in men despite the results of a recent study with no significant effects of soy protein or isoflavone intake indicating that regardless of dose and study duration, neither soy protein nor isoflavone exposure affects total testosterone (TT), testosterone (FT), estradiol (E_2) or estrone (E_1) levels in men (Reed et al. 2021).

In addition to being a good source of calcium and milk protein, SMP 34% was included as a functional ingredient utilized as a natural nutraceutical filler to improve the texture and taste and ensuring shelf life stability. Vitamins (folic acid, B_{12}, C), minerals (iron, zinc), steviol glycosides, and natural flavors (vanilla, cinnamon, coffee, chocolate) were added in effective concentrations, and all the formulations were processed and packaged in sealed metallized polypropylene (MPP) sachets.

The usage of nutritional supplements among athletes has significantly expanded, both to improve performance and to satisfy dietary needs. Therefore, adulterated products emerged on the market, with serious side effects such as strokes, acute liver injury, kidney failure, and even death. To identify the adulteration of crucial products, numerous analytical assessments were devised (Hashem et al. 2020; Filho et al. 2022; Martins et al. 2022).

85% of the total milk used to make cheese is discarded as whey, which is rich in valuable components, especially lactose sugar and soluble proteins like beta-lactoglobulin (β-Lg) (40–50%), alpha-lactalbumin (α -La) (12–15%), bovine serum albumin (BSA) (5%), and immunoglobulins (8%) with high contents of essential amino acids from which are the valuable branched chain amino acids (BCAAs) with high digestibility (Gantumur et al. 2023). As a reliable and reasonably priced carrier and stabilizer of bioactive compounds, WPC has been employed for encapsulation. In comparison to maltodextrin or Arabic gum, it provides higher protection for bioactive compounds (Ji et al. 2022). Whey protein before breakfast may improve postprandial glucose excursions in centrally obese individuals after fasting low-moderate intensity exercise without affecting hunger or subsequent calorie intake (Allerton et al. 2021). WPC 70% was used over the other forms (isolate or hydrolysate) as it is well-documented the superior characteristics of whey protein concentrate with more bioactive components than the other forms. WPCs are currently used as fat mimetics in reduced-fat formulations (Lammert et al. 2014).

Vanillin, the molecule responsible for the flavor of vanilla, is extracted from vanilla pods, making vanilla, the "Queen" of spices, one of the most widely used flavors in the food and cosmetics sectors worldwide (Wilde et al. 2019). The antioxidative, anti-apoptotic, anti-inflammatory, neuroprotective, and anticancer properties of vanillin are well documented. Antioxidant, antiproliferative, depressive, and anti-glycating effects are all present in vanilla. Doxo-induced toxicity in H9c2 cardiac cells is lessened by vanilla. Cardiomyocytes could be shielded by vanillin against doxo-induced cell damage. Vanillin was applied therapeutically to mitigate the cardiotoxicity caused by anthracyclines and enhance the long-term efficacy of antineoplastic therapy (Sirangelo et al. 2020).

Cinnamon, due to its wide range of bioactive phenolic compounds, including catechin, protocatechuic acid, quercetin, epicatechin, p-coumaric acid, p-hydroxybenzoic acid, syringic

acid, rosmarinic acid, caffeic acid, ferulic acid, and chlorogenic acid, has been used in traditional medicine for its protective role. These compounds have therapeutic effects against inflammation, oxidative stress, diabetes, obesity, hypertension, hypercholeste (Akilen et al. 2013; Das et al. 2022). Due to its functional compound, which also lowers the risk of cancer, hyperlipidemia, and hyperglycemia and has anti-bacterial and anti-tyrosinase activities, cinnamon has been shown to have antimicrobial properties in clinical studies. It is also used as a natural anti-browning additive in the active packaging materials (Senevirathne et al. 2022; Siew et al. 2022).

Coffee contains more than 1000 chemical compounds, from which the biological effects of coffee are due to caffeine, chlorogenic acid, trigonelline, cafestol, kahweol, and ferulic acid (Surma and Oparel 2021). Caffeinated coffee intake increased to a moderate level (1–7 cups per week) in an elderly Mediterranean cohort at elevated cardiovascular risk, but not at higher levels. linked with a decrease in visceral adipose tissue (VAT), trunk fat, and overall body fat. Decaffeinated coffee was not related to adiposity indicators. In an elderly population with obesity, moderate caffeine consumption may be a weight-management approach (Henn et al. 2023).

Cocoa powder; a rich source of flavonoids has a beneficial role in lipid metabolism due to its polyphenols, reducing the risk of coronary heart disease (CHD), reducing blood pressure (BP), and increasing plasma antioxidant capacity (Khan et al. 2012). Numerous cognitive outcomes are positively impacted by cocoa consumption. These positive benefits appear to be accompanied by a rise in cerebral blood flow or cerebral blood oxygenation following cocoa consumption. Young people who consumed cocoa flavanols on a regular basis showed improved cognitive function and higher levels of neurotrophins (Martín et al., 2020). Flavanols, which are a type of flavonoid, are abundant in cocoa. The antioxidant and anti-inflammatory properties of these substances are what give cocoa its health-promoting properties. Cocoa polyphenols interact in both directions with the gut microbiota once they enter the intestine. These substances have the ability to alter the gut microbiota's makeup through prebiotic mechanisms. They inhibit the development of pathogenic bacteria like *Clostridium perfringens* while promoting the growth of beneficial gut bacteria like *Lactobacillus* and *Bifidobacterium*. Contrarily, bioactive cocoa metabolites can improve gut health by acting as anti-inflammatory agents, boosting immunity, and lowering the chance of a number of diseases (Sorrenti et al. 2020).

Stevia leaves extract contains steviol glycosides (SGs), chlorogenic acids, caffeoylquinic acids, and dicaffeoylquinic acids, with great health benefits, antioxidant, antibacterial, antiviral, and anti-inflammatory effects (Ai et al. 2022; Moongngarm et al. 2022). Stevia preparation display health-promoting effects, especially the antidiabetic action. SGs are the compounds responsible for the sweet taste of stevia (Gardana and Simonetti 2018; Molina-Calle et al. 2016). SGs have anti-inflammatory, oral health-promoting, anti-hypertensive, and cancer-prevention properties. Additionally, they assist in controlling blood sugar levels by influencing glucose uptake, enhancing insulin production, or raising the concentration of glucose transporters (Kurek and Krejpcio 2019).

Conclusions

In the context of nutraceuticals, which are essential in improving quality of life (QoL) for a variety of populations, including those with non-communicable diseases, malnutrition, and specialized nutritional needs, the current study tackles a significant issue. The significance of well produced formulations built on a scientific foundation is emphasized throughout the text. In order to guarantee that nutraceuticals are efficient and safe to consume, this is crucial. It's exciting to think of biological synergism between whey protein concentrate and steviol glycosides. It implies that utilizing these two elements together may have a more significant effect than using them alone, which may be of interest to researchers and dietary product producers. Overall, the essay discusses a nutraceuticals-related issue that could have significant implications. It was determined that all formulations were of the highest quality and safety, with the greatest customer acceptance being recorded in the following order: chocolate > coffee > cinnamon > vanilla-flavored formulations.

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